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Seroepidemiological Study of Respiratory Syncytial Virus in São Paulo State, Brazil

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Transmission of respiratory syncytial virus is thought to be highly seasonal based on reported clinical cases, although transmission resulting in mild disease in all age groups has been little studied. This has been investigated in a seroepidemiological survey using sera from São Paulo, Brazil. Seroprevalence was found to increase rapidly with age, reaching over 90% by three years of age. This is typical of viral infections, which produce life-long immunity following primary infection. One-hundred percent seropositivity was attained by five years of age and maintained throughout adulthood, whereas mean antibody titers continued to increase with age. The mean duration of maternal antibodies was estimated to be 3.3 months with antibody decay demonstrated in paired samples from infants. The results are discussed in relation to possible mechanisms generating such a profile. ***J. Med. Virol. 55:234–239, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: epidemiology; transmission; infection; antibody; seasonality; ELISA

INTRODUCTION

Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract disease in infants worldwide and responsible for significant morbidity in adults, particularly the elderly [Collins et al., 1996]. However, despite its undoubted important contribution to the global burden of disease, relatively little is understood of the transmission dynamics of this infection. Most studies to date have concentrated on the epidemiology of clinical disease, rather than infection, so that, given the inherent biases in hospital samples, there can be few conclusions drawn from these studies regarding community-based transmission. For example, it is well known that clinical RSV disease is highly seasonal in most epidemiological settings, resulting in large epidemics in short periods of time [Brandt et al., 1973; Kim et al., 1973; Mufson et al., 1973; Winter and Inglis, 1987], as was typically seen for measles prior to immunization programs [Anderson and May, 1991]. With respect to measles, epidemics are driven by numbers of susceptibles that are depleted following an epidemic through development of a long-lasting protective immune response after primary infection. Yet repeat infection with RSV is common despite previous exposure, which may suggest that transmission should not have a strong seasonal pattern. As yet, community-based studies have not addressed the issue of seasonal transmission, so that this remains an apparent paradox.

Much of the understanding of virus transmission dynamics is based on childhood infections spread by close contact (e.g., measles, mumps, and rubella). These viruses generally exist as single (antigenic) strains, and primary infection tends to result in an immune response that is protective to subsequent infection. This allows the development of relatively simple models of transmission and consequent development of statistical techniques to analyze antibody prevalence and mathematical models to aid design of vaccination programs [Anderson and May, 1991]. In this context, age-specific serological surveys have proven invaluable in furthering the understanding of the transmission of virus.

By contrast, it is not clear what the presence of antibody to RSV implies. It is clear that they do not result in protection against infection, at least against heterologous strains, and the age profile of disease would suggest that they are protective against severe disease (rather than infection), although, again, this is largely speculation. It is also known that a substantial proportion of infants experiencing their first RSV infection (who are hospitalized) do not show a rise in antibody

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titer [Murphy et al., 1986; Cane et al., 1996; Brandenburg et al., 1997], suggesting that lack of antibodies is not always an indication of no previous exposure.

To date, there have been no seroepidemiological studies of RSV reflecting age-specific patterns of antibody prevalence in communities using sera collected specifically for epidemiological purposes. Indeed, although serological studies have been carried out reflecting acquisition of antibody in infants [Glezen et al., 1986], there have been few cross-sectional serosurveillance studies published, particularly in developing countries [Brüssow et al., 1991]. Seroepidemiological studies in a variety of geographical settings may give a broader understanding of community-based RSV transmission patterns, and enable a clearer interpretation of the effect of antibody status on infection and disease. Such information is crucial for the design of cost-effective control programs.

In this study sera were screened for IgG antibodies to RSV to further investigate the dynamics of infection within a suburban population. The sera were collected solely for the purposes of seroepidemiology and thus reflect the patterns of acquisition of antibody in the general population.

TABLE I. Age-Specific Sample Sizes of Sera Taken in the Initial Survey (December 1990)*

Age class	Number of sera	Repeat
0 m (cord)	39	0
1–2 m	27	20
3–4 m	22	18
5–6 m	27	23
7–8 m	48	41
9–10 m	37	25
11–12 m	27	20
13–18 m	29	25
19–23 m	25	19
2 y	50	0
3 y	44	0
4 y	33	0
5 y	14	0
6–10 y	35	0
11–15 y	12	0
16–20 y	20	0
21–30 y	43	0
31–40 y	17	0
Total	549	191

*Including the maternal: cord pairs, and those for which a sample was taken exactly one month later (January 1991). Age in months (m) and years (y).

METHODS AND MATERIALS

Collection of Sera

The study used samples collected in 1990 and 1991 from the town of Caieiras, in northern São Paulo, Brazil, for the investigation of rubella seroprevalence. The collection of sera as well as demographic details and methods used for sampling the community have been described previously [Azevedo Neto et al., 1994]. The study population was chosen as representative of a suburban Brazilian community. Standard theory [Cochran, 1977] was applied to achieve a random two-level cluster sample from families within randomly selected administrative regions. Rubella vaccine was administered to all children under two years of age and a second blood sample collected exactly one month later to assess seroconversion. The age-structure of the samples used in this study are presented in Table I. These include 471 sera from all ages in the initial community survey, 191 repeat samples from rubella vaccinees, and 39 maternal:cord pairs collected from Hospital Regional de Caieiras (EMED). Sera separated from blood samples were stored at –20°C.

Immunoassay

RSV strain A2, for use as antigen, was cultured in BSC-1 cells to give maximum cytopathic effect (CPE). Cells were harvested by scraping and sedimented by centrifugation. Cells were resuspended in distilled water (DW) with 0.5% NP40 and debris removed by centrifugation. Control antigen was prepared in an identical way from uninfected cells.

Antigen (or control) was coated onto microtiter plates (Immulon II, Dynatech, USA), at optimal dilution determined by checkerboard, in DW and allowed to evaporate dry. Plates were then fixed using acetone containing 20% DW for 10 minutes and then air-dried. Plates were blocked with 3% skim milk powder (SMP) in PBS pH7.4 for 30 minutes at 37°C, then washed three times using PBS plus 0.05% Tween20. Sera was added at 1/200 in PBS-SMP for two hours at 37°C to test and control wells. After washing three times, antihuman IgG horseradish peroxidase conjugate (Dako, Denmark) was added at 1/2000. After incubation for two hours at 37°C and washing, orthophenylamine diamine (OPD) substrate was added, stopped with 2-M H_2SO_4, and plates were read at 490 nm.

Data Analysis

All plates included serial dilutions (neat to 1/128) of a high-titer local standard of pooled adult sera to generate a standard curve. This was given arbitrary unit value of 1,000. All test sera were calibrated using the standard curve and antibody reported as $\log_{10}$ units. Determination of an appropriate cutoff delineating positive and negative sera was assessed using a frequency distribution of antibody titers for all sera screened. Paired sera were screened in adjacent wells on the same assay plate. Data was analyzed using Corel Quattro Pro 7 (Corel Corporation Ltd., Corel, Canada) and SPSS release 6.0 (SPSS Inc., USA). The mean rate of maternal antibody decay (d) was estimated using linear regression on log antibody concentrations, assuming

$$x_a = x_o.e^{-d.a}$$

where x_a represents the mean antibody titer of infants with maternal antibodies at age a and x_o the mean antibody titer at birth.

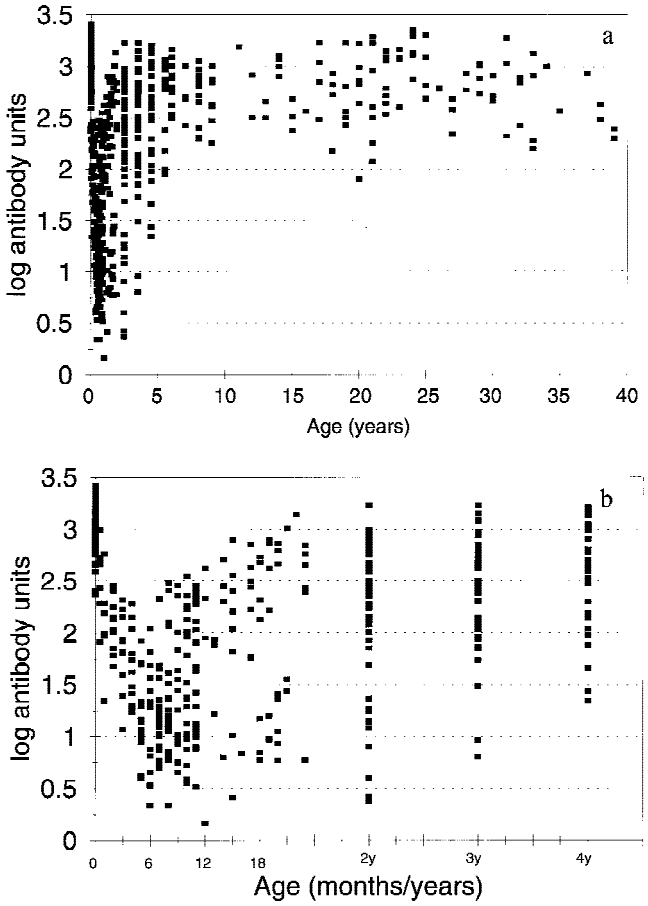

Fig. 1. Scatter plots of antibody levels for all samples (n = 549) in the initial survey by age. The two graphs depict samples for all ages **(A)** and 0–4 years **(B).**

RESULTS

Antibody titers for all samples in the first survey (n = 549) by age are shown in Figure 1, which depicts more clearly the titers for different age groups. Figure 1A, 0 to 40 years, shows that all individuals above the age of five had high levels of antibody and these are maintained throughout adulthood. Figure 1B, for the age range of 0–4 years, illustrates the decline in maternally derived antibody during the first six months after birth. The mean duration of maternal antibody was calculated at around 3.3 months (1/d; d = 0.306). After the age of six months there were clearly higher antibody levels in some individuals, presumably as a result of primary RSV infection and the proportion seronegative decreases. Antibody titers in those infected appear to increase after nine months of age.

A cutoff between positive and negative sera could therefore be deduced from the scatter plot, particularly between the ages of 12 and 24 months, and would fall within the range of 1.5 and 2 log units. This was confirmed from the bimodal frequency distribution of antibody titers for all sera (Fig. 2). The mid-point between distributions of negative and positive sera (1.7 log units) was used as the cutoff point for seropositivity.

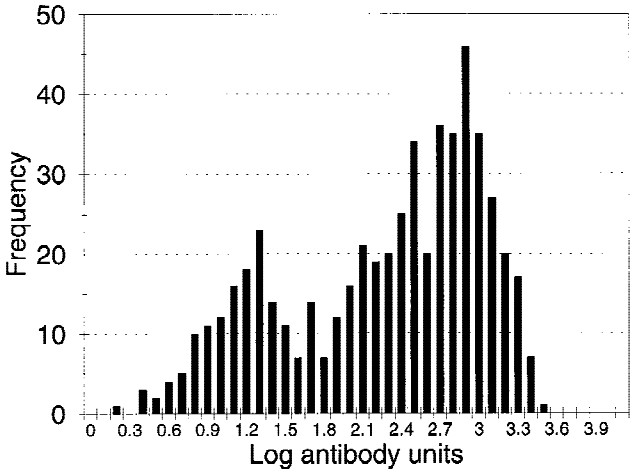

Fig. 2. Frequency distribution for all samples (both surveys n = 740) used to delineate between those seropositive and seronegative for RSV antibodies estimated at around 1.7 (log units).

Using this cutoff, an age–serological profile can be constructed, as seen in Figure 3. The decline in maternal antibody was rapid after two months of age, with the highest proportion of infants seronegative for RSV between six and eight months of age. Thereafter, the proportion seropositive rises rapidly with age, such that 90% have had their first infection by three years of age and 100% by five years of age. The proportion seropositive remains at 100% throughout adulthood. The mean antibody titers, in those who are seropositive, for different age groups are presented in Figure 4. There was a rapid decrease in mean maternal antibody titer during the first six months; thereafter antibody levels were seen to rise rapidly and significantly between six and 24 months and then more slowly thereafter through to adulthood. Mean antibody levels in cord sera (0 months) were clearly higher than in the adult age groups.

Analysis of maternal:cord serum pairs (Fig. 5) revealed a significant correlation between antibody titer for each pair (n = 39, r = 0.854, $P < 0.0001$). The mean titers in cord sera (x = 3.0078, standard error (SE) = 0.042) were found to be significantly higher (paired differences, t = 5.01, degrees of freedom (df) = 38, $P < 0.0001$) than those in maternal sera (x = 2.88, SE = 0.047).

Results from paired samples from infants aged between one and 24 months of age are shown in Figure 6. Variation in antibody titer between samples was likely due to a number of reasons, including factors during the assay procedure. For the majority of samples there was little change in antibody titer between collection of the sample pairs. However, two samples did appear to have large increases in IgG titer, possibly the result of recent infection. A few infants over six months of age showed decreases in antibody titer but the majority of seropositive paired samples showing a decrease were from infants below six months of age, reflecting the decline in maternally derived antibody during the one-month period.

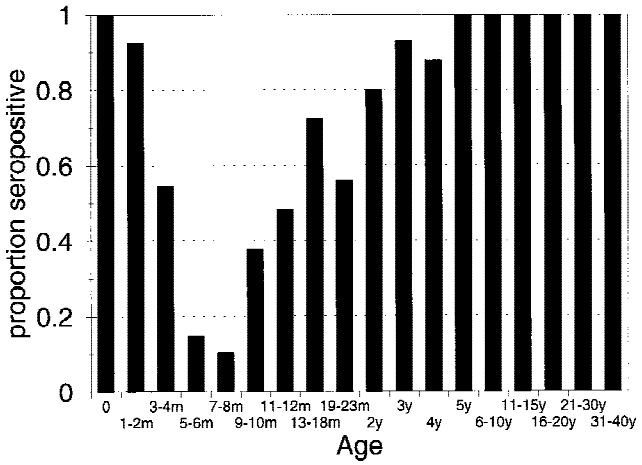

Fig. 3. Age seroprevalence profile with the proportion seropositive (n = 549) against age in months (m) and years (y) (see Table 1 for sample sizes) using a cutoff for seropositivity defined using the bimodal distribution in Figure 2.

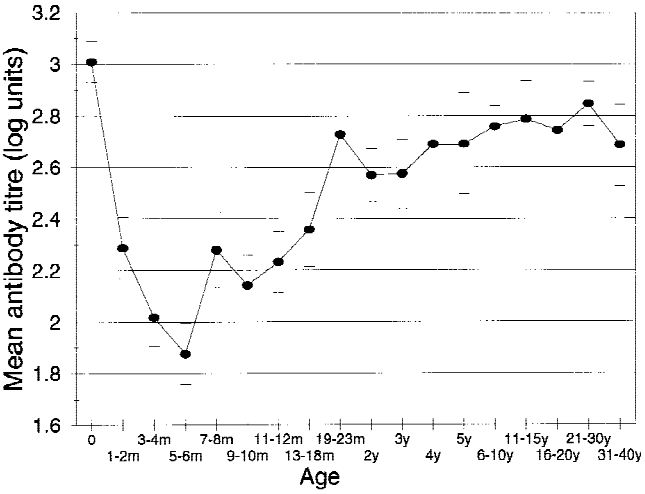

Fig. 4. Mean antibody levels for all samples defined as seropositive (n = 398) by age (95% confidence intervals). Age in months (m) and years (y).

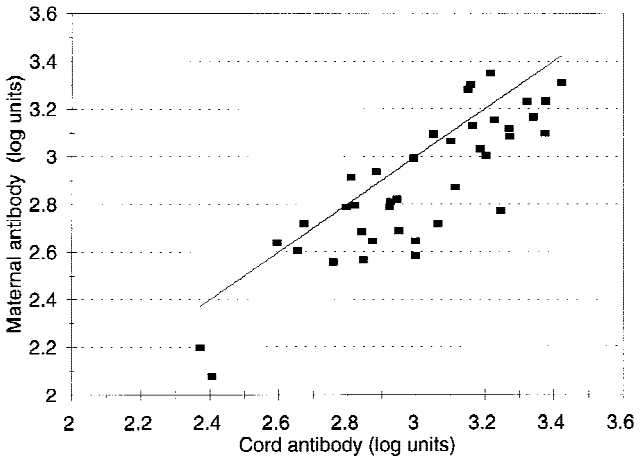

Fig. 5. A comparison of antibody titers in maternal:cord pairs.

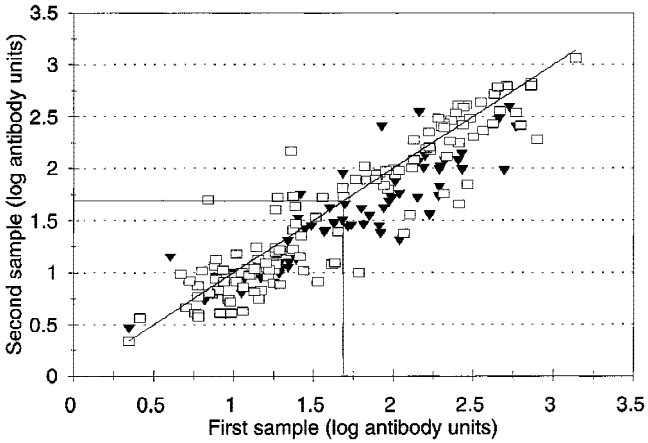

Fig. 6. A comparison of antibody titers in paired sera (n = 192) from infants aged between one and 24 months over a one-month period (December 1990 to January 1991). ▼, represents individuals aged one to six months; □, aged between seven and 24 months.

DISCUSSION

A simple enzyme immunoassay (EIA) technique was used to screen sera quantitatively for RSV IgG. This has been applied to a large community-based study of individuals selected randomly with respect to infection and disease status.

Epidemiologically, a number of important observations can be made from the patterns shown. Despite the comprehensive differences between RSV and single-strain childhood viruses, the age-related pattern presented in Figure 3 appears remarkably similar to those typically observed for measles, mumps, and rubella. Maternally derived antibodies (Fig. 5) decay with a half-life of 3–3.5 months, which is typical of other viral infections, including measles [Anderson and May, 1991], mumps [Cox et al., 1989], and rubella [Nokes et al., 1986]. All cord sera were seropositive, and the results clearly demonstrated the correlation between RSV antibody levels in maternal and cord sera, with the average levels of antibody in cord sera significantly higher due to active placental IgG transport [Kohler and Farr, 1966]. Dependant on titer, maternally derived RSV antibody has a protective effect against clinical infection, being rare in infants less than one month but protection is rapidly lost [Holberg et al., 1991].

The rise in prevalence of antibodies (Fig. 3), presumably due to infection, from the age of eight months onward shows that exposure to RSV is frequent, with the majority of infants having primary exposure to RSV by two to three years of age [Collins et al., 1996]. Complete seropositivity in adults has been reported [Johnson et al., 1961] with antibody levels higher than those seen in infants [Anderson and Heilman, 1995]. Following the loss of maternally derived antibody, antibody titers in those seropositive continue to increase throughout all the age groups, with significant changes seen in the first two years. The continued increase could be explained by a number of processes. In infants, develop-

ment of immune competence [Murphy et al., 1986] may explain the rapid increase in the first couple of years. Indeed, the increase in the proportion seropositive is likely to be an underestimate of the average age at primary infection, given that a proportion of infants do not IgG-seroconvert upon first exposure. However, it is clear that all infants seroconvert at some time and it is not known what proportion do not upon first exposure and whether this is related to infection outcome. Certainly studies that report the lack of seroconversion are referring to severe disease and this does not necessarily negate the value of seroepidemiology as has recently been suggested [Brandenburg et al., 1997].

Repeat exposure to virus leading to subclinical or clinical infection probably has an important role in boosting antibody responses throughout life. In other viral infections (e.g., rubella and mumps) [Nokes et al., 1986; Cox et al., 1989], a significant decay in mean antibody titers with age throughout adulthood has been observed, most likely due to reduced exposure to virus in older age classes and the development of a protective immune response upon primary exposure. The continued increase in antibody titers into adulthood provides some evidence that reexposure to, and possibly reinfection with, RSV was relatively common. This would provide a substantial source of virus, which may account for the rapid increase in prevalence early in life. An explanation for rapid infection may be that a significant proportion of adults are infected and shedding virus during the epidemic season.

A constant supply of susceptibles (of all ages) would predict that transmission of infection would be more continuous rather than epidemic. Clinical epidemics could be generated by strain variation. Certainly there appears to be variability in virulence between genotypes [Walsh et al., 1997], although epidemics are known to consist of multiple genotypes [Cane and Pringle, 1991] and of differing proportions [Cane et al., 1994].

Interpretation of the profiles presented in this study must consider possible seasonal variation of RSV transmission in Brazil, where clinical cases have been shown to peak between February and July [Nascimento et al., 1995]. If this were also true for 1990, then infants younger than six months were less likely to have been exposed to infection. The rapid increase in seropositivity after eight months of age could reflect high infection rates during the previous high transmission season of younger infants. A cross-sectional survey conducted at a different time may have revealed a different profile, confirming epidemic transmission.

Given the confusing relationship between antibody status, strain variation, and reinfection, it is clear that longitudinal studies are required for investigation of the transmission dynamics of RSV. The development of mathematical models of multistrain viruses [White et al., in press] will also be required to interpret the seroepidemiology of these infections and enable the impact of control programs to be assessed and predicted.

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